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SCHIZOSACCHAROMYCES OCTOSPORUS

W. C. Coker and Louise Wilson

(With Plate 55, Containing 25 Figures)

In the course of some special work on wild and domestic yeasts during the fall of last year (1910) we were surprised to discover in Chapel Hill a member of the rare genus *Schizosaccharomyces* Lindner. The species appeared in the following manner: Unbroken Delaware grapes bought in the Chapel Hill market were put in a test tube, covered with distilled water, and set aside. Slow fermentation started up and a precipitate began to appear. After three weeks, an examination of the precipitate showed all stages of development, both vegetative and reproductive. A close examination showed the species to be *Schizosaccharomyces octosporus* Beyerinck. Cultures were made from this in various solutions and in most cases spore formation was secured. Fresh cultures of this yeast were also obtained later from California Tokay grapes, using the same method as with the Delaware grapes.

The genus *Schizosaccharomyces* is a small one proposed by Lindner* in 1893 to include those forms of conjugating yeast-like fungi which multiply vegetatively not by budding, as in other yeasts, but by fission, as in most cell division. The species *S. Pombe* was the first to be discovered and is the one on which the genus was founded. Since that time three other species of the genus have been described: *S. octosporus* Beyerinck,† *S. mellacei* (Jörg.) Lindner,‡ and *S. asporus* Eyckmann. Of these, *S. Pombe* and *S. mellacei* produce only four spores in conjugation, while in *S. asporus,* as the name implies, spore formation seems to be suppressed.

The members of this genus form a peculiar group of yeasts hitherto found only in hot countries, and so far little known. *S. mellacei* was found in Jamaica, *S. Pombe* in tropical Africa,

* Wochenschr. f. Braueri **10**: 1298. 1893.

† Centralblatt f. Bacteriol. **16**: 49.

‡ Mikr. Betriebs-kontr. Berl. 404. 1901.

and *S. octosporus* on old currants presumably from Greece. The appearance of the last at Chapel Hill is the first reported occurrence, so far as we know, of any of the genus on the American continent. Schiönning* was the first to publish observations on the conjugation of *S. octosporus.* He found that the cells destined to form the ascus divide into daughter cells which do not separate completely, but remain attached at a certain point. After some time the partition at the point of attachment is absorbed and the two sister cells fuse to form an ascus with eight spores.

Guilliermond† in 1903 confirmed these observations in large part but thought that while the fusion may take place simply by the solution of the partition wall it is generally brought about by the formation of two short projections just above the point of contact, which meet at their tips and fuse.‡

Guilliermond also studied the nuclear behavior and found that the two nuclei fuse in conjugation and then divide into eight daughter nuclei around which the eight spores are formed. This, then, is to all appearances a true case of isogamous sexual reproduction. In a later paper§ Guilliermond reports further observations on this yeast and thinks he has exaggerated a little in saying that it is always sister cells that fuse. From certain appearances, which he figures, he thinks that the fusing cells may occasionally be separated from each other by several cell divisions.

In our study of the development of *Schizosaccharomyces octosporus,* extending over several months, we largely confirmed the observations of the authors above mentioned. However, we saw no indication whatever of fusion between any but sister cells, nor any sign of conjugation through projecting tubes or processes. In plate LV, we give a series of drawings showing conjugation (figs. 14 to 25). The condition shown in figs. 17, 18 and 25 is not due in our opinion to the fusion of elongated processes, but to the drawing out of the cells after fusion. The typical series is perhaps best represented by figures 14, 15, 16, 20 and 21. Where the cells divide in vegetative reproduction they do not break away all along the line, but bend back and remain attached by one corner. After a time they break away entirely and repeat the process. Figs. 1 to 10 show a reproductive series of forms and stages in ordinary growth and multiplication.

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* Compt. Rend. des Brau. Lab. Carlsberg. 4: 1895. We have not been able to see this paper.

† Rev. Gen. de Bot. 15: 1903.

‡ This method reminds us of the conjugation in certain species of *Spirogyra* where neighboring cells may fuse by such a projection.

§ Rev. Gen. de Bot. 17: 337. 1905.

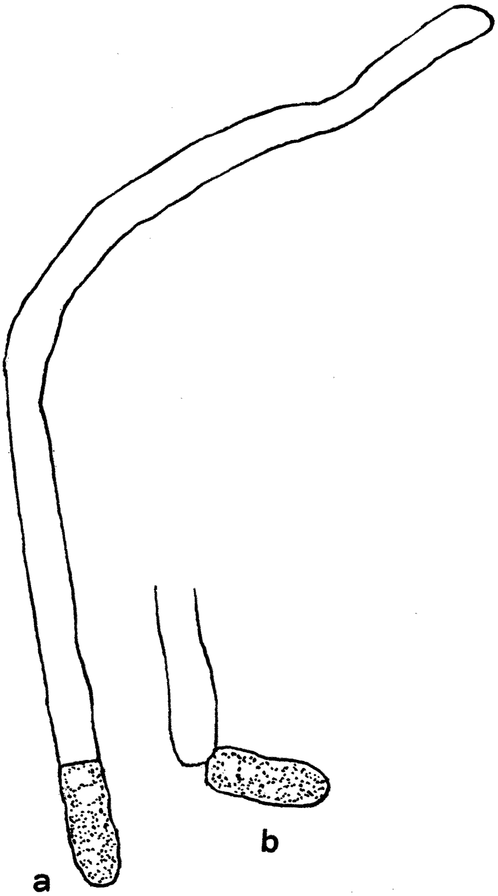


FIG. 1. An elongated hypha of *S. octosporus* cutting off a normal cell at its tip. × 670.

Even in normal vegetative growth the size of the cell varies considerably, and when food begins to fail many of the cells extend their growth into elongated hyphae which formed a conspicuous part of the mass. In fig. 13 is shown the beginning of such a hypha. As the hypha elongates the protoplasm is usually confined to one end, where growth alone proceeds. After a time this protoplasm collects more closely into the tip and is cut off by a cross wall. This tip cell then bends around just as in ordinary division, and remains for a time attached by a point. Later it drops away and becomes a vegetative cell of normal size. In text fig. 1a, such a hypha is shown with its protoplasm cut off at one end. While this figure was being drawn the end cell was seen to bend back as shown in text fig. 1 b. Occasionally there may be seen long rows of cells as shown in text fig. 2 recalling certain figures of Lindner's for *S. Pombe*. These finally break up into separate cells.

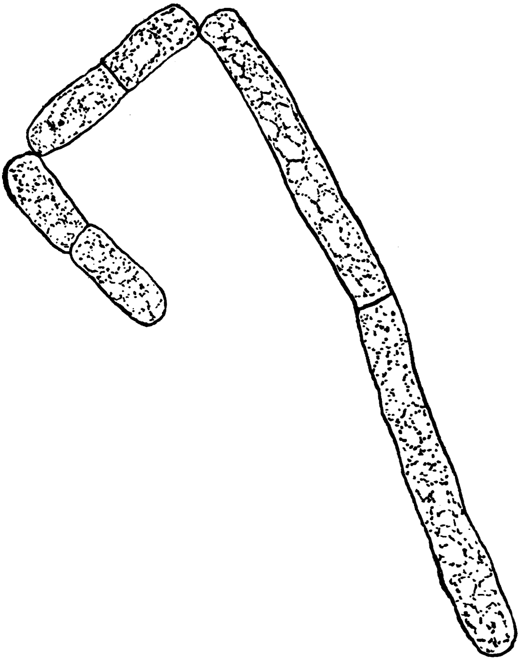
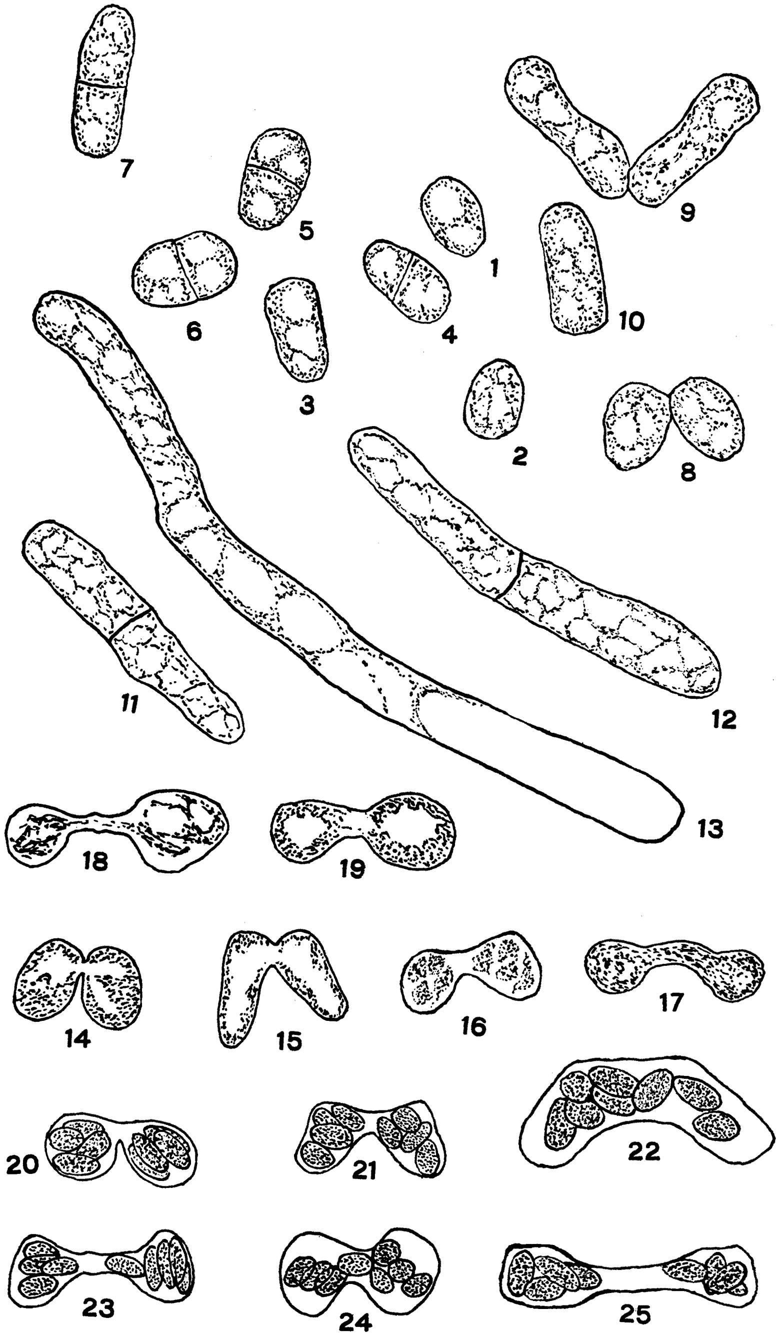


FIG. 2. Elongated cells of *S. octosporus* in rows beginning to separate. × 1080.

When fusion is to take place the procedure is exactly the same as in vegetation cell division, up to the point of the bending back of the cells. Then, instead of separating, the partition becomes absorbed and the contents of the two cells fuse. Usually, the original position of the cell is retained and the result is the formation of a double sac that resembles a pair of saddle bags (figs.



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20, 21). This shape may be variously modified as shown in the figures. A dumbbell form is frequently seen.

In a recent paper* Guilliermond gives a good review of our present knowledge of spore formation, in the yeasts. Among them sexuality is known to appear in two ways. In *Schizosaccharomyces, Zygosaccharomyces,* and *Debaryomyces,* there is a fusion of cells before spore formation. In *Saccharomyces Ludwigii, Willia Saturnus,* and *yeast of Johannisberg II,* there is no fusion preceding the formation of spores, but the spores fuse during the act of sprouting. In a number of other cases spores are formed but there is no conjugation either before or after.

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EXPLANATION OF PLATE 55

Schizosaccharomyces octosporus Beyerinck

Figures 1–11. Normal stages of vegetative growth and division. × 1080.

Figures 12, 13. Elongated forms showing the beginning of hyphal development. × 1080.

Figures 14–25. Stages in conjugation and spore formation. × 1080.

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* Ann. Myc. 8: 287. 1910.